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A Complex, Five Breakpoint Intrachromosomal Rearrangement Ascertained Through Two Recombinant Offspring

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Intrachromosomal rearrangements usually result from three or fewer breaks. We report a complex intrachromosomal rearrangement resulting from five breaks in one chromosome 10 of a phenotypically normal father of two developmentally delayed children. GTG-banding analysis of the father's rearranged chromosome 10 suggested an initial pericentric inversion followed by an insertion from the short arm into the terminal band of the long arm [der(10)(pter→p13::q21.2→p12.2::q22.1→q26.3::q22.1→q21.2::p12.2→p13::q26.3→qter)]. To our knowledge, this rearrangement is the most complex ever reported in a single chromosome. Both children inherited a recombinant chromosome 10 with loss of the insertion and the segment distal to it [rec(10)der(pter→p13::q21.2→p12.2::q22.1→q26.3:)]. Mechanisms for both rearrangements are proposed.

KEY WORDS: chromosome 10, intrachromosomal rearrangement, inversion, insertion, recombinant chromosome, CCR

INTRODUCTION

Most balanced intrachromosomal rearrangements can be classified as either inversions or insertions. Inversions result from two breaks in a chromosome. Intrachromosomal insertions, or "shifts," require three breaks and are less frequently reported. A constitutional rearrangement involving more than three breakpoints in a single chromosome has been described previously in only one case [Romain et al., 1985]. We report an apparently balanced, intrachromosomal rearrangement which appears to have resulted from five breaks in one chromosome 10. The rearranged chromosome 10 was found in the phenotypically normal father of two developmentally delayed children. Both children inherited a recombinant chromosome which had interstitial segments of both short and long arms, as well as the terminal segment of the long arm, deleted.

CASE REPORTS

A family with two children was seen for counseling because of mild developmental delay in both children. Two additional pregnancies had ended in an ectopic gestation and in an elective termination due to hyperemesis.

The older child was born at term by cesarean section because of failure of labor progression. His birth weight was 3,210 g and the Apgar scores were 4 and 7 at 1 and 5 minutes, respectively. During the first year no significant difficulties were noted. He walked at 1 year of age and, soon after, the development of speech and fine motor skills was noted to be delayed. At 2½ years of age, patchy vitiligo was noted on the torso which progressively expanded. Awkward movements with slight ataxia, mild conductive hearing loss, and mild mental retardation also became apparent. MRI of the brain did not show abnormalities. Physical examination, at 9 years and 4 months of age, revealed a height of 143 cm (95th centile), weight of 30.2 kg (50th–75th centile), head circumference of 57 cm (greater than the 98th centile), broad-based gait, slight ataxia, marked vitiligo with large amelanotic areas over the entire body except for the forearms, thighs, and part of the face, synophrys, long eyelashes, increased vascularity of the conjunctiva, mild arachnodactyly of the fingers, asymmetry of the hands with the left side longer than the right (right 3rd finger 7 cm, left 3rd finger 8 cm), marked arachnodactyly of the toes, and small testicles (<1.5 cm).

The younger child, a female, was born by cesarean section at 38 weeks gestation after a pregnancy complicated by gestational diabetes. Cesarean delivery was difficult, requiring an incision through the pla-

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centa which was complicated by fetal blood loss resulting in an anemic newborn. Birth weight was 3,230 g and Apgar scores were 2 and 8 at 1 and 5 minutes, respectively. At birth she was pale and hypotonic, and had mild respiratory distress and a left hip dislocation. At 9 months of age, at the time of our initial evaluation, she was described by her mother as being floppy and having difficulty with visual focusing. A Denver Developmental Assessment was normal except for a delay in the fine motor skills. Physical examination showed that her height was 73.4 cm (75th–90th centile), weight was 8.14 kg (25th centile), and head circumference was 44.3 cm (50th–75th centile). She had generalized mild hypotonia with hypoactive reflexes, synophrys, patent anterior fontanelle (2.2 cm), prominent metopic suture, convergent strabismus, receding chin, and bilateral deformity of the 2nd and 3rd toes with extension of the proximal and flexion of the distal joint (hammer toe deformity). The skin revealed no pigmentary alterations.

Cytogenetic Studies and Interpretation

Chromosome analysis was performed on peripheral blood lymphocytes from both patients and their parents using GTG-banding [Seabright 1971]. One paternal chromosome 10 had an abnormal banding pattern suggestive of a partially deleted short arm and an additional dark band at the terminus of the long arm (Fig. 1). The rearranged chromosome 10 was interpreted to be the result of an initial pericentric inversion (Fig. 2a) followed by an intrachromosomal insertion into the terminal band of the long arm. The rearrangement would be designated der(10)(pter→ p13::q21.2→p12.2::q22.1→q26.3::q22.1→q21.2::p12.2→p13::q26.3→qter) [ISCN (1995)]. The inserted segment was partially derived from the short arm and partially from long arm material resulting from the inversion (Fig. 2b). The segment derived from the short arm inserted directly with respect to the centromere, while there was inverted insertion of the segment derived from the long arm. However, the long arm segment after insertion was returned to its proper orientation with regard to the centromere due to the proposed inversion.

Both children inherited a chromosome 10 which was identical in appearance to the abnormal paternal chromosome except that the dark band at the terminus of the long arm was deleted (Fig. 1). The abnormal chromosome 10 in the children is probably a recombinant [rec(10)der(10)(pter→p13::q21.2→p12.2::q22.1→q26.3:)] (Fig. 2c). Maternal chromosomes were normal.

Fluorescence in situ hybridization (FISH) with a "painting" probe specific for chromosome 10 (Coatasome™ 10 purchased from Oncor and used according to the instructions of the manufacturer) was used to rule out a cryptic translocation. The probe hybridized along the entire length of both the normal and rearranged chromosomes 10 of the father and the children indicating that the abnormal chromosomes were derived entirely from chromosome 10 (data not shown). The probe did not hybridize to any other chromosomes.

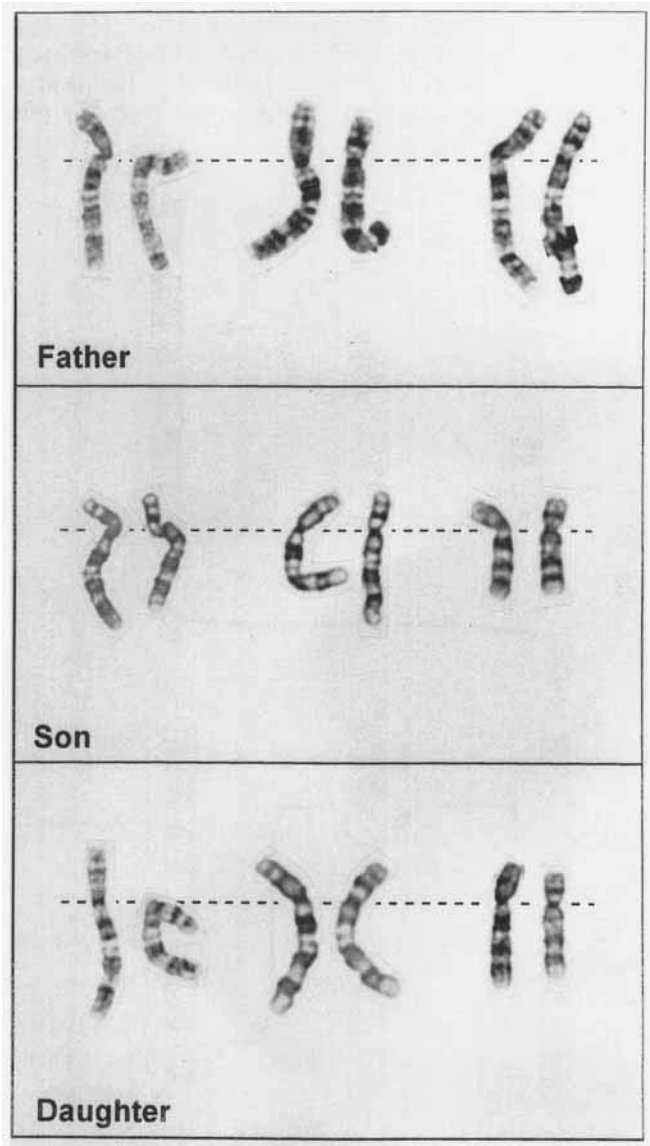

Fig. 1. The chromosome 10 homologues from three metaphase cells of the father and the children. The abnormal chromosome 10 in each pair is on the right.

Both children had been karyotyped previously at other centers. The karyotype of the older child had been reported as 46,XY,10p-. An amniocentesis karyotype of the younger sibling performed by a different laboratory was reported as normal.

DISCUSSION

Our analysis of the abnormal paternal chromosome 10 suggests that five breaks occurred, two resulting in the pericentric inversion and three additional breaks involved in the insertion. On the other hand, if the segment which shifted to the long arm actually fused with the telomere instead of inserting into the terminal band, the rearrangement would require only four breaks. We were unable to resolve this question with

certainty, although the appearance of the abnormal chromosome in some GTG-banded metaphases supported the former interpretation. However, fusion of an interstitial band from one chromosome with the telomere of a second chromosome has been reported [Reeve et al. 1993].

The accuracy of our cytogenetic interpretation could be tested using FISH with probes hybridizing to the segments of chromosome 10 involved in the insertion (p12.2→p13 and q21.2→q22.1). However, we have been unable to perform this work because the sequence specific probes currently available for hybridization to those chromosomal regions require freshly prepared slides and the family has been uncooperative in providing repeat specimens.

Although both the inversion and the insertion may have originated in the father, it is possible that the rearrangements occurred over multiple generations. The inversion may have occurred in an earlier generation and the insertion in a subsequent generation. Chromosome analysis of the paternal grandparents might have clarified this; however, they were unavailable for study.

Figure 2c illustrates a proposed mechanism for the formation during spermatogenesis of the recombinant chromosome found in the children. Three complete loops and one hairpin loop are hypothesized for meiotic synapsis of the normal and abnormal paternal homologues. The recombinant chromosome is proposed to have arisen from a single or any uneven number of crossovers within the hairpin loop (q22.1→q26.3) ("LMNOPQ" in Fig. 2c). A crossover anywhere within this segment would result in the same recombinant chromosome with deletion of the insertion and the segment of the long arm distal to it. The reciprocal recombinant from this crossover would have duplication of these same segments. It is not known whether complete synapsis occurs in the case of a chromosome with such a complex rearrangement. However, synapsis of just the q22.1→q26.3 segment of the long arm followed by crossover would produce the same recombinants.

The recombinant chromosome is unusual because of the concurrent deletion of interstitial segments of both long and short arms plus deletion of the terminal band of the long arm. Crossovers in any of the other three loops of the pachytene configuration would result in much greater imbalance and would probably not produce live-born offspring.

We have interpreted the rearrangement in the father, who is phenotypically normal, as balanced, and that of the children as unbalanced. Yet, despite the deletion of several chromosome bands, neither of the children showed severe structural malformations or severe mental retardation. A possible explanation is that the deleted segments either contain mostly non-coding DNA sequences or lack "critical" genes.

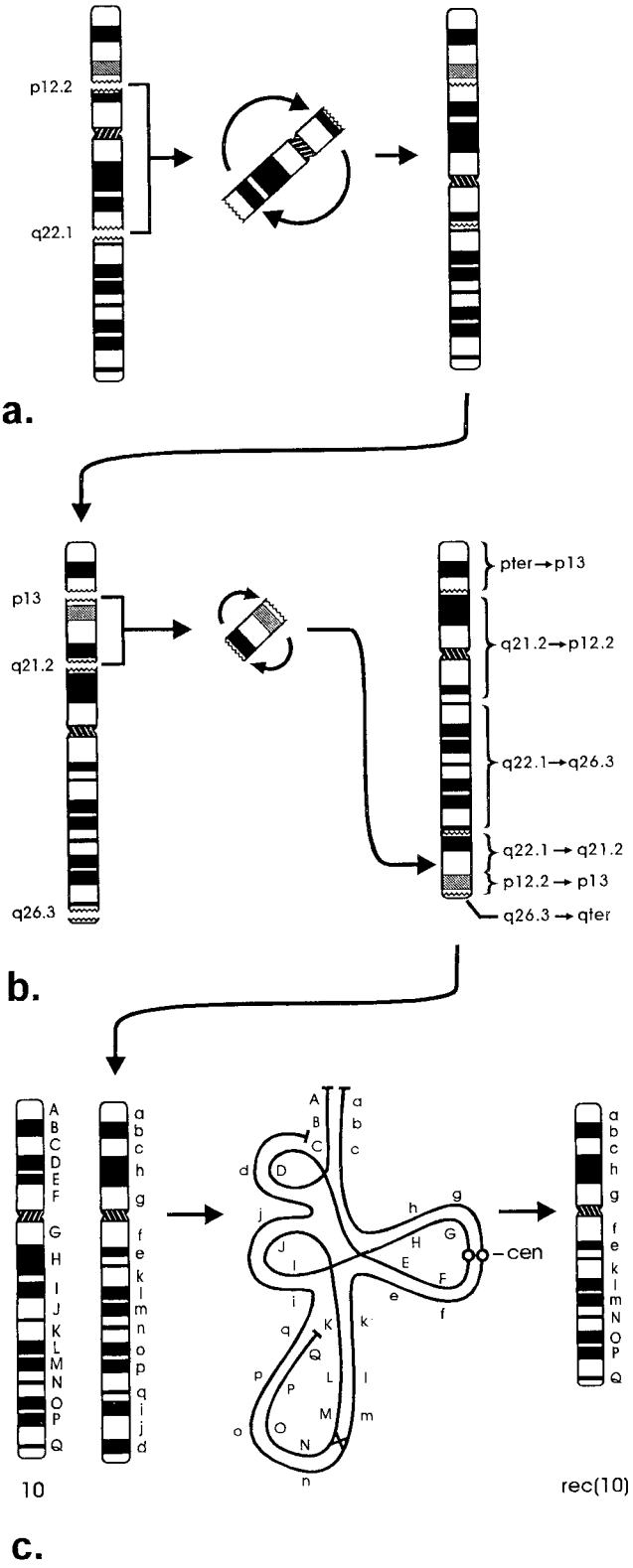

Fig. 2. Proposed origin of the abnormal paternal chromosome 10 (a and b) and the recombinant chromosome 10 of the children (c). **a:** Pericentric inversion in one paternal chromosome 10. **b:** "Shift" of a segment from the short arm of the inverted chromosome 10 into the terminal band of the long arm; note that the inserted segment is partially derived from short arm material and partially from long arm material resulting from the inversion. **c:** The normal and rearranged paternal chromosomes 10 (left) synapse in a complex pachytene configuration during meiosis (center). Crossing over between the normal and rearranged chromosomes resulted in the recombinant 10 inherited by the children (right).

It is also of interest that although both children appear to have a similar cytogenetic anomaly, the clinical findings are different. This might be ascribed to age or sex differences; however, other explanations are feasible. For example, a translocation between the paternal chromosome 10 homologues could give rise to a derivative chromosome similar in appearance to the children's abnormal chromosome. If the abnormal chromosomes in the children resulted from different mechanisms of formation (i.e., one recombinant and one derivative), then different complements of alleles would be present in the children's abnormal chromosomes. The children's phenotypic differences could be the result of such differences in gene complement. However, since the likelihood of translocation presumably would not be increased by the presence of the paternal rearranged chromosome, a translocation seems unlikely. Another possible explanation is that the two children inherited different maternal chromosomes 10 and are, therefore, hemizygous for different alleles in the region of the deletion. Although phenotypic variation such as is seen here is not typically encountered with deletion syndromes having well-characterized phenotypes presumably resulting from loss of critical genes or where imprinting may be involved, it may well be the case with deletions in which the missing segment is hypothesized to contain few "critical" genes.

Most intrachromosomal rearrangements fall into the categories of inversions or insertions which require two or three breaks, respectively. Rearrangements resulting from more than three breaks in a single chromosome have been reported rarely. The case described by Romain et al. [1985] involved a pericentric inversion and an interstitial deletion in a single chromosome 4. Two alternative interpretations of the rearrangement were presented by the authors; one proposed four breakpoints and the other only three. The patient had relatively few structural malformations and mental retardation was mild.

Our review of the literature revealed no other cases of intrachromosomal rearrangement with more than three breaks. It is likely, however, that such rearrangements are under-reported. Balanced rearrangements, such as the paternal rearrangement reported here, may never be ascertained in the absence of unbalanced offspring or altered fertility. Even if ascertained, such anomalies may be inaccurately interpreted (e.g., 10p- reported in the initial chromosome study of the older sibling described here). Since characterization of a chromosomal rearrangement becomes more difficult as the number of breaks increases, some multi-breakpoint anomalies may go unreported because of difficulty in interpreting the rearrangement. Finally, some intrachromosomal rearrangements may simply be missed, as was the recombinant 10 of the younger sibling reported here when studied prenatally.

Although complex chromosomal rearrangements (CCRs) are relatively common as acquired abnormalities in neoplastic tissues, they are rarely found as constitutional anomalies [reviewed by Batista et al., 1993]. CCRs were originally defined as rearrangements resulting from more than two chromosome breaks and involving three or more chromosomes [Pai et al., 1980; Gardner and Sutherland, 1989]. ISCN (1995) uses examples with a minimum of four breakpoints in two or more chromosomes. The case presented here, which involves five breakpoints in a single chromosome, although certainly complex, would not meet these criteria for CCRs. Therefore, we suggest that the definition of CCR be broadened to include this and other similar cases.

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